SURE_J: Science Undergraduate Research Journal

Volume 4 | Issue 1 Article 2

December 2022

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Cover Page Footnote

I would like to thank my academic supervisor, Dr. Lisa Geraghty at the Technological University of the Shannon, whose expertise has been extremely valuable throughout the duration of this project. In particular, I want to thank you for your ongoing input and guidance throughout. Secondly, I would like to thank the Technological University of the Shannon for the use of the facilities during my time spent here.

Science Undergraduate Research Experience Journal *SURE-J*
Volume (XX), Issue (XX)

Anthelmintic Resistance in Sheep Across Ireland and the UK: a literature review of the *in vivo* versus *in vitro* methods

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Received xxxxxx, Accepted for publication xxxxxx, Published xxxxxx

Abstract

Background: The animal welfare and production consequences of gastrointestinal nematode infections, namely *Nematodirus battus*, on sheep are compelling. The control of gastrointestinal infections has been heavily reliant on the administration of anthelmintics since their introduction into mainstream use in 1960. However, their frequent and often excessive use has resulted in anthelmintic resistance being reported extensively in several gastrointestinal nematode species. The prevalence of anthelmintic resistance as well as the increase in the number of cases of multiple-drug resistance in nematode populations now poses substantial hindrance on the viability of sheep production systems. However, despite the threat *N. battus* poses to the sustainability of sheep farming, there are very few publications on this nematode.

Aim: The aim of this study is to investigate the prevalence and epidemiology of *N. battus* in sheep across Ireland and the United Kingdom between the years 2011 and 2021. Relevant studies were reviewed from the literature to identify *N. battus* resistance to the benzimidazole and macrocyclic lactone anthelmintic groups and to assess anthelmintic efficacy using *in vivo* and *in vitro* methods. Preliminary data was also gathered from the distribution of a survey to identify the perceived efficacy of anthelmintic treatments and the management factors that may be contributing to anthelmintic resistance. The dissemination of the survey was primarily shared via social media platforms.

Results: The results obtained from the literature highlighted variation in the epidemiology of *N. battus.* Although the historically proposed lifecycle which occurs during the spring period is prevalent across Ireland and the UK, clinical nematodirosis in autumn is becoming more frequent. Resistance to a number of the broad-spectrum anthelmintics was identified in 60% (n=10) of the publications from the UK, with the benzimidazoles being reported as the least effective. The assessment of anthelmintic efficacy highlighted that there is a lack of effectiveness and sensitivity in the *in vivo* and *in vitro* methods that are readily applied in field conditions. From the 134 responses obtained from the survey, a number of concerns were raised with management practices. It was concluded that many flock owners have become complacent with incorrect dosing and a lack of calibrating of dosing equipment, as seen in 69% and 63% respectively. In addition, the prevalence of *N. battus* is becoming more evident on farms across Ireland and the UK, with 62% of farms having an outbreak of *N. battus* infection on the farm within the last five years. Although many of these respondents identified clinical signs in lambs during the predicted spring period, a number of respondents (6.7%)

proposed that infection occurred during the autumn period, which concurred with previous findings.

Conclusion: The extensive use of anthelmintics on sheep production systems has led to the rapid progression of anthelmintic resistance across Ireland and the UK, particularly to the broad-spectrum anthelmintics. Without the use of effective and reliable testing methods and the implementation of stringent monitoring strategies, the fight against anthelmintic resistance will be a losing battle. It is critical that there is more emphasis on the need for improving worming strategies on sheep farms. Without effective worm control and a resistance management flock plan, animal health and anthelmintic efficacy will continue to deteriorate.

Keywords: Gastrointestinal nematodes, anthelmintic resistance, *Nematodirus battus*, multiple-drug resistance, sheep, sustainability, prevalence, epidemiology, Ireland, United Kingdom, *in vivo, in vitro.*

1. Introduction

Gastrointestinal nematode (GIN) infections pose substantial constraint on several ovine production systems, consequently resulting in economic and production losses (McRae, *et al.,* 2015). In particular, the roundworm *Nematodirus battus*, has constituted a major welfare burden for sheep-farming across Ireland and the United Kingdom (UK) (Dempsey, 2019). Infection with *N. battus* is a major challenge to naïve and vulnerable livestock, particularly young lambs. Acute disease, more commonly known as nematodirosis, is common in lambs 6 to 12 weeks of age, resulting in ill-thrift, and the rapid onset of profuse watery diarrhoea, thus, resulting in dehydration and in severe cases, mortality (Dempsey, 2019).

Since being first reported in 1951, *N. battus* has become endemic in several parts of Europe and other temperate regions across the northern hemisphere (Rodríguez–Vivas, *et al.*, 2017). Many studies have indicated the high seasonality of *N. battus* (Melville, *et al.*, 2020). It has been hypothesised that acute disease arises during the months of late spring and early summer (Melville, *et al.,* 2020). Despite the high rate of contamination on pastures during this period, eggs that are deposited on pastures from livestock will not hatch into infective larvae until the next grazing season, affecting the following lamb crop, see figure 1.1 (Melville, *et al.*, 2020; Boag and Thomas 1975). Although it is believed that the winter/freezing stimulus has been a vital aspect of the hatching of *N. battus*, the characteristic seasonal dynamics of parasite epidemiology is evolving (McMahon, *et al.*, 2012). Recent reports have demonstrated changes in egg population hatching and pasture abundance without chilling (Melville, *et al.,* 2020; Van Dijk and Morgan, 2010; Van Dijk and Morgan, 2008). Although the exact causes of these epidemiological phenomena are unknown, there are however, a number of proposals for the selective pressures on this GIN; from changes in farm practices and climate change, to variations in host availability and spring temperatures (McMahon, *et al.*, 2012; Van Dijk and Morgan, 2010).

For over 60 years, the administration of anthelmintics has been a vital constituent in controlling GIN infections in sheep (Vineer, *et al.,* 2020; Coles, *et al.,* 2006). While anthelmintics have traditionally aided in the control of helminth infections for many years, they have been used excessively, resulting in anthelmintic resistance being reported in several GIN species. These efficacy issues are particularly evident among the broad-spectrum anthelmintics including the benzimidazoles (BNZ) and the macrocyclic lactones (ML) (Keegan, *et al.,* 2017; Rose, *et al.,* 2015; Papadopoulos, *et al.,* 2012). The prevalence of anthelmintic resistance as well as the increase in the number of cases of multiple-drug resistance in nematode populations is a compelling animal health concern and now poses substantial hindrance on the viability of small-ruminant production systems.

Despite the threat *N. battus* poses to the sustainability of small-ruminant production systems, there are very few publications on the prevalence of this nematode. As a result, this study aimed to investigate the prevalence and epidemiology of *N. battus* in sheep across Ireland and the UK between a ten-year period. Relevant studies were reviewed from the literature to identify *N. battus* resistance to the BNZ and ML anthelmintic groups and to assess anthelmintic efficacy using *in vivo* and *in vitro* methods. Preliminary data was also gathered through the use of a survey to identify the perceived efficacy of anthelmintic treatments and the management factors that may be contributing to anthelmintic resistance.

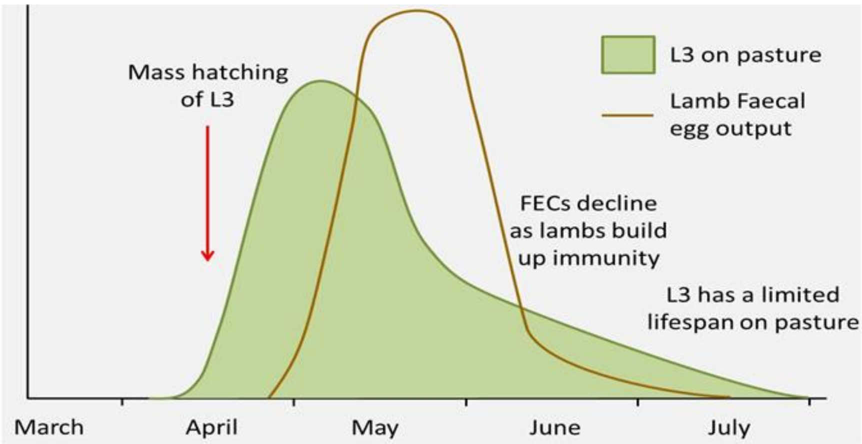

Figure 1.1: The historically proposed lifecycle of *N. battus* (Farm Health Online, 2018).

2. Methods

2.1 Literature review

A systematic review was carried out in accordance with the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) guidelines between December 2021 to February 2022 (Moher, *et al.*, 2009). The literature search consisted of using a collection of databases, including EUR-Lex, PubMed (Medline), ScienceDirect, BSAVA, and publications available through the Technological University of the Shannon library to obtain quantitative data.

Study inclusion criteria

To obtain information regarding the prevalence of *N. battus*, the resistance status to the broad-spectrum anthelmintics and anthelmintic efficacy using *in vivo* and *in vitro* methods, a study was regarded eligible if: (i) it stated the author(s) name, (ii) it was carried out in Ireland or the UK, (iii) it was published between 2011-2021, (iv) it was written and published in the English language, (v) the sample size was ≥15, (vi) it stated the number of positive, susceptible or resistant cases, (vii) it stated the study type, either cross-sectional or longitudinal, (viii) it stated the host, (ix) it stated the GIN nematode species identified, and, (x) it identified anthelmintic resistance or anthelmintic treatment failure in sheep.

Data extraction and analysis

Once the screening of data was complete, the next step involved extracting the data from the relevant studies using the PICO (Population, Intervention, Comparison, and Outcomes) model, see table 2.1. The full body of the study along with the reference list was reviewed by a single reviewer. The extracted data included the title of the study, the name of the author(s), the period in which the study was conducted, the country in which the study took place, the year of publication, the host, the use of *in vivo* and/or *in vitro* methods, the sample size and positive recordings, and the GIN species under review. All the admissible information was analysed regarding the effectiveness of the methodology which included recording its validity, reliability, and sensitivity as well as all statistical analysis used.

Table 2.1: Keyword search for the population, intervention, and outcome for the study.

Acronym	Keyword search
Population	(Sheep OR ovine OR Ovis aries OR small ruminants OR lamb OR Ireland OR United Kingdom OR northern hemisphere)
Intervention	(Exposure OR breed OR refugia OR association OR prevalence OR survey OR management factors OR alternatives OR targeted selective treatment OR farm management OR pasture management OR age OR calibration OR dosage OR drench and move OR frequency OR route of administration)
Outcome	(Broad spectrum anthelmintic OR benzimidazole OR albendazole OR fenbendazole OR oxfenbendazole OR macrocyclic lactone OR ivermectin OR moxidectin OR milbemycin OR nematode OR gastrointestinal nematode OR roundworm OR *Nematodirus battus* OR parasite OR resistant OR resistance OR anthelmintic resistance in sheep UK OR anthelmintic resistance in sheep Ireland OR *in vivo* tests OR *in vitro* tests)

2.2 Survey design

A survey was distributed to the wider farming community across Ireland and the UK through the use of three social media platforms for one week during February 2022. All responses were gathered and collated using the Microsoft forms platform. Ethical approval was granted by the Technological University of the Shannon.

Question selection

The survey was developed consisting of 29 questions, which was divided into two sections: (i) general farm details and (ii) worming regime and grazing management, see appendix 1. Questions referring to anthelmintic treatments and anthelmintic failure on the farm were included to establish the estimated prevalence of anthelmintic resistance in these flock. Similarly, questions concerning the management practices on the farm, as well as the technical aspects of the dosing equipment and calibration, were included to establish any potential correlation between these factors and anthelmintic resistance. Questions requiring any personal or sensitive information were removed to ensure confidentiality and comply with Regulation (EU) 2016/679 (General Data Protection Regulation).

Piloting

A pilot study of the survey was conducted by three farmers. This test allowed for any unclear language to be rectified, and any problems were reported.

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Analysis of data
The survey window was limited to one week, after which, the survey was deactivated to ensure no more data was collected. The data generated were exported to an Excel spreadsheet where it was analysed and further broken down to obtain the relevant information.

3. Results

3.1 Literature review results

Relevant studies reported between the years 2011 and 2021 were reviewed, all of which are summarised below in tables 3.1, 3.2 and 3.3. The data gathered from these literature studies were divided into three main categories.

3.1.1 Prevalence of *Nematodirus battus* across Ireland and the UK

Despite the threat *N. battus* poses to the health of livestock, very little information can be found on this nematode in the literature. A systematic review was carried out to establish the prevalence and epidemiology of *N. battus* across Ireland and the UK via post-mortem and faecal egg count data, see table 3.1.

The percentage of positive samples of *N. battus* in sheep (based on submissions to the Department of Agriculture, Food and the Marine in Ireland and Agri-Food and Biosciences Institute in the UK) were considerably higher in the UK than in Ireland during the periods of 2011-2016. In particular, there were considerable differences in 2013, 2014 and 2015, where the percentage of positive samples were 16%, 11% and 16% higher sequentially, see figure 3.1 (AFBI and DAFM, 2015; AFBI and DAFM, 2014; AFBI and DAFM, 2013).

In Ireland, the percentage of samples reported with *N. battus* during 2018 was 14.5% (n=1673). The classic highly seasonal peak was observed in May and June, with 24% and 32% of samples testing positive, respectively. However, from these positive samples, 820 tested positive for *N. battus* during the autumn period; September (17%), October (17%) and November (15%). Similarly, faecal samples obtained from the Republic of Ireland during 2019 also displayed similar results. However, it was observed that the percentage of positive samples during the September and October period were greater than those in April, by 4.1% and 3.1% sequentially.

Table 3.1: Raw data from the 'All-island Animal Surveillance Report' based on the percentage [%] of positive samples diagnosed with *N. battus* infection from 2011-2021 across Ireland and the UK.

Year	Country	Positive Samples [%]
2011	Ireland	5
	UK	16
2012	Ireland	1.6
	UK	9
2013	Ireland	5
	UK	21
2014	Ireland	5
	UK	16
2015	Ireland	6
	UK	22
2016	Ireland	10
	UK	16
2017	Ireland	14
	UK	8.6
2018	Ireland	14.5
	UK	8
2019	Ireland	10.7
	UK	4.6
2020	Ireland	11
	UK	8.4

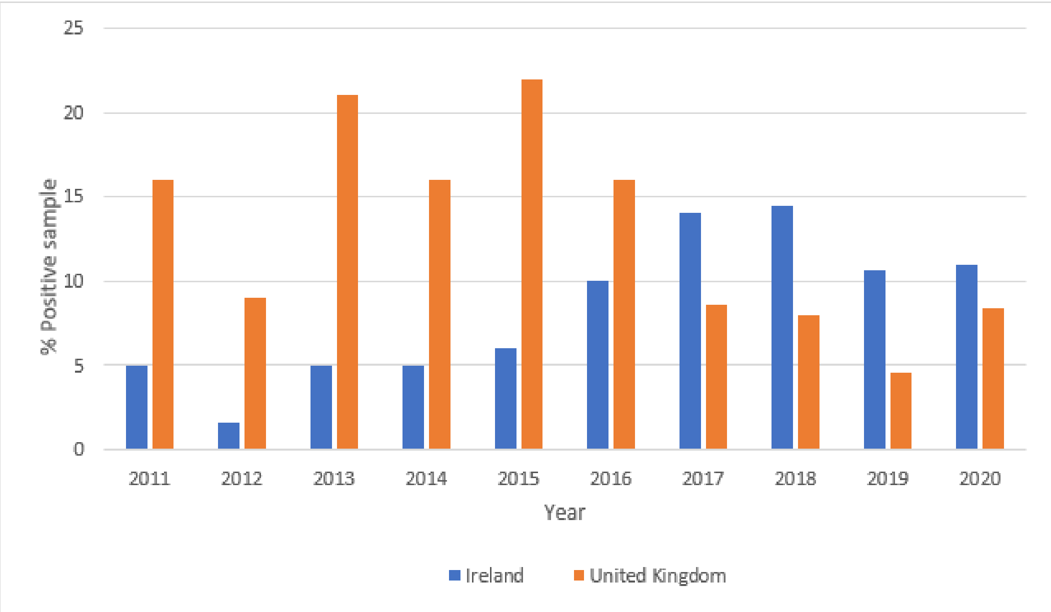

Figure 3.1: Distribution of the percentage [%] of positive samples diagnosed with *N. battus* infection from 2011-2021 across Ireland and the UK.

3.1.2 Identification of *Nematodirus battus* resistance to the benzimidazole and macrocyclic lactone anthelmintic groups across Ireland and the UK

Based on the data obtained from the literature, 60% of the publications (n=10) identified resistance to a number of the broad-spectrum anthelmintics in *N. battus* populations, all of which were from the UK. From the limited number of studies published in Ireland, anthelmintic resistance was rarely recorded in the BNZ and the ML.

Table 3.2: Summary of studies between 2011-2021 used to assess *N. battus* resistance to the benzimidazole and macrocyclic lactones in sheep across Ireland and the UK.

Authors	Country	Method of diagnosis	Anthelmintic Class	Factors reported
Mitchell, *et al.*, (2011)	United Kingdom	Farm-based (FECRT)	BNZ	First report of BNZ resistance in *N. battus.*
Melville, *et al.*, (2020)	United Kingdom	Molecular analysis	BNZ	Resistant single-nucleotide polymorphism (SNPs) in *N. battus* identified. Additionally, the first case of the early emergence of the mutation (F167Y SNP) was established.
Morrison, *et al.*, (2014)	United Kingdom	Controlled Efficacy Test (CET) & Molecular analysis	BNZ	Resistance to the BNZ, fenbendazole, reported in *N. battus.*
Jones, *et al.*, (2012)	United Kingdom	Farm-based	ML	No suspicion of ML resistance in *Nematodirus spp.*
Keane, *et al.*, (2018)	Ireland	Molecular analysis	N/A	The research conducted did not identify substantial levels of SNPs associated with resistance. There was nominal resistance associated with *N. battus* with SNPs on OAR 3, 4, 7 and 12.
Matika, *et al.*, (2011)	United Kingdom	Molecular analysis	N/A	The results indicated positive results. Quantitative trait loci were established on chromosome 3 for *N. battus* in purebred Texel and Suffolk sheep.
Riggio, *et al.*, (2013)	United Kingdom	Molecular analysis	N/A	This study showed positive results in the identification of SNPs on chromosome 14 for *N. battus* in Blackface lambs.

McMahon, *et al.*, (2017)	United Kingdom	Farm-based	BNZ, ML	The study identified anthelmintic resistance in *N. battus* populations in the UK. Resistance to BNZ, and the MLs, moxidectin and avermectin were present in 36%, 75% and 50% of flocks tested.
Keegan, *et al., (*2017)	Ireland	Farm-based	BNZ, ML	From the 338 drench tests carried out for *N. battus* populations, the overall treatment efficacy was established to be 96%.
Keane, *et al.*, (2014)	Ireland	Farm-based	BNZ, ML	Anthelmintic resistance was rarely recorded. BNZ was 100% effective (n=48), meanwhile, ML was 94% effective (n=47).

3.1.3 Anthelmintic efficacy across Ireland and the UK using *in vivo* and *in vitro* methods in gastrointestinal nematodes

The efficacy of the faecal egg count reduction test (FECRT) was analysed in 44% of the studies obtained from the literature, see table 3.3. Of note, comparison studies were carried out exclusively in two separate studies by Keane, *et al.*, (2014) and McMahon, *et al.*, (2013). In both studies, the FECRT was deemed effective in detecting anthelmintic resistance to the BNZ and ML across a number of GIN species.

The comparison of the efficacy of the *in vivo* FECRT to *in vitro* tests, including the larval development assay (LDA), controlled efficacy test (CET) and the larval migration inhibition assay (LMIA) was also conducted by Keegan, *et al.*, (2015). Here, the use of the standardised FECRT was carried out to assess the resistance status in lambs artifically infected with ivermectin resistant *Teladorsagia circumcincta* to BNZ, and ivermectin. The FECRT proved effective in confirming resistance to all three anthelmintics. In contrast, a CET was carried out to assess its agreement with the FECRT and to determine the effectiveness of the ML, moxidectin. In accordance with the FECRT, the CET confirmed resistance to ivermectin. Additionally, the LDA confirmed resistance to BNZ, meanwhile the LMIA confirmed resistance to ivermectin and indicated moxidectin susceptibility in the isolates. All three *in vitro* tests confirmed the results obtained during the FECRT.

Based on the data obtained from the literature, approximately 55% of the studies (n=9) suggests that the use of the molecular analysis is highly efficacious in identifying genomic regions associated with resistance in GIN (Melville, *et al.*, 2020; Morrison, *et al.*, 2014; Riggio, *et al.*, 2013; Matika, *et al.*, 2011).

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Table 3.3: Summary of studies between 2011-2021 used to assess the anthelmintic efficacy of *in vivo* and *in vitro* methods in gastrointestinal nematodes in sheep across Ireland and the UK.

Authors	**Country**	**Tests**	**Factors reported**
Ramünke, *et al.*, (2016)	Ireland	FECRT, Egg Hatch assay (EHA) & Molecular tests	The use of pyrosequencing for the detection of BNZ resistance in *Haemonchus, Teladorsagia* and *Trichostrongylus* showed to be more sensitive than FECRT's and/or EHA.
McMahon, *et al.*, (2013)	United Kingdom	FECRT	The distribution of anthelmintic resistance was observed on various sheep farms across the UK. A high level of resistance, 81%, was detected in flocks using BNZ (n=24); and the MLs, avermectin 50% (n=7) and milbemycin 62% (n=13).
Keegan, *et al.*, (2015)	Ireland	FECRT, CET, LDA & LMIA	This report identified the first case of ivermectin resistance and multiple-drug resistance in sheep in Ireland. It was identified by the FECRT and the CET that two isolates of *Teladorsagia circumcincta* were resistant to BNZ, and ML ivermectin. However, resistance was not detected for the ML moxidectin. The LDA detected resistance in the isolates to BNZ. In addition, the LMIA identified ivermectin resistance and also indicated susceptibility to moxidectin.
Keane, *et al.*, (2014)	Ireland	FECRT	Based on 369 flock FECRT's, only 51% of anthelmintic drenches were deemed effective.
Keane, *et al.*, (2018)	Ireland	Molecular tests	It was concluded that although loci associated with nematode resistance have been established in previous studies; this research did not conform. As a result, the paper concluded that the use of SNP x breed interaction is not a consistent marker analysis.
Matika, *et al.*, (2011)	United Kingdom	Molecular tests	The research conducted indicated positive results in accordance with previous studies. Quantitative trait loci were established on chromosome 3 for *N. battus* and strongyles genera in Texel and Suffolk sheep. This study gives rise to the potential of applying this technology to crossbred progeny in the future.
Riggio, *et al.*, (2013)	United Kingdom	Molecular tests	The resistance in GIN was analysed in Blackface lambs using genome-wide association and regional heritability mapping approaches. Both methods performed well during the identification of resistance. In particular, similar regions for SNPs on chromosome 14 were identified for *Nematodirus.*

Melville, *et al.*, (2020)	United Kingdom	Molecular tests	Resistant SNPs in *N. battus* were identified. Additionally, the first case of the early emergence of the mutation (F167Y SNP) was also established at a low frequency using molecular analysis.
Morrison, *et al.*, (2014)	United Kingdom	CET & Molecular tests	Resistance to the BNZ, fenbendazole reported in *N. battus*. Evidence recorded suggests that the mutation in the β-tubulin isotype-1 gene is a likely mechanism of resistance in *N. battus*, but further work is needed.

3.2 Survey of worming practice and perception of anthelmintic resistance

A total of 134 completed surveys were received, from which, 111 respondents were from Ireland and 23 from the UK, see figure 3.2. The survey was designed to collect data on the perceived prevalence of *N. battus* across Ireland and the UK as well as identify the perceived efficacy of anthelmintic treatments and the management factors that may be contributing to anthelmintic resistance.

It was concluded that many farms either did not have an issue with roundworms (n=63) or did not know if roundworms were an issue on the farm (n=21). Despite this, 87% (n=117) still administered anthelmintic drugs to control roundworms; with the BNZ (n=77) and the ML (n=43) being the most frequently used within the last 12 months. Only a small number of farms used faecal egg counts (n=36) or obtained advice from a veterinarian (n=10) to assess when to administer anthelmintics to livestock. Out of the 134 respondents, 82% treated all of the lambs, meanwhile only 18% targeted those showing signs of ill-thrift.

According to the guidelines outlined by SCOPS, livestock should be dosed “at the rate that is recommended for the heaviest in the group” (SCOPS, 2020). This guideline was upheld in 37% of flocks. The second most common means of determining the volume of drug to be administered included obtaining the group average weight on 31% of farms, however, this incorrection calculation of dose can lead to over or underdosing livestock (SCOPS 2020; McMahon, *et al.*, 2017; McMahon, *et al.*, 2013). Additionally, 37% of flock owners only sometimes or never calibrated their dosing equipment.

It was concluded that 62% of farms had an outbreak of *N. battus* infection on the farm within the last five years. A number of respondents (6.7%) proposed that infection occurred during the autumn period, which concurred with previous reports of clinical nematodirosis occuring during the autumn period (Melville, *et al.*, 2020; Sargison, *et al.*, 2012).

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Figure 3.2: Compilation of the counties that the data were gathered from during the survey.

4. Discussion

4.1.1 Prevalence of *Nematodirus battus* across Ireland and the UK

In recent years, the epidemiology of *N. battus* appears to be shifting. Stark variations were identified in the percentage of positive samples recorded for *N. battus* infection when they occurred in the UK and Ireland from 2011 to 2021. Although a substantial reduction has occurred following this, particularly during 2019, the data still proposes that *N. battus* is considerably more prevalent in the UK, than across Ireland. The seasonality and abundance of this GIN firmly corresponds to extrinsic factors such as the climate and the management of pastures (Sargison, *et al.*, 2012). Traditionally, it has been proposed that the lifecycle of *N. battus* eggs requires a prolonged period of chilling in order for hatching to occur (AFBI and DAFM, 2015; Boag & Thomas, 1975). Contrary to this, more recent studies are being conducted to assess the variations in the hatching of *N. battus,* where infection has been observed during spring, and also during the autumn period (Melville, *et al.*, 2020; Sargison, *et al.*, 2012). The changing trend of a second autumnal peak in nematodirosis infection can be demonstrated during 2018 and 2019. The observation of the changes in the lifecycle of *N. battus* is not a new concept as this shift in pattern was identified by Van Dijk, *et al.*, (2008). Although reports of clinical nematodirosis during the autumn period have become more prevalent, the changes in the pattern of events are generally unclear.

It has been hypothesised that unpredicted hatching of *N. battus* during the autumn period occurs due to a proportion of eggs being shed in the spring without the prior chilling stimulus as normally anticipated (AFBI and DAFM, 2019; Sargison, *et al.*, 2012). Given modern and intensive sheep farming practices, these synchronised hatchings occurring both earlier and later in the year can coincide with the presence of susceptible and naïve lambs on pastures.

Although these findings did concur with recent studies, it is clear that on-going analysis should be carried out in order to assess its implications on the health of livestock.

4.1.2 Identification of *Nematodirus battus* resistance to the benzimidazole and macrocyclic lactone anthelmintic groups across Ireland and the UK

The first case of BNZ resistance in *N. battus* populations was detected on a farm within the UK in 2010 (Mitchell, *et al.,* 2011). Following the use of the *in vivo* faecal egg count reduction test (FECRT), resistance to BNZ was confirmed after the treatment with fenbendazole only proved to be 83% effective, hence, it did not meet the 95% reduction criteria (Mitchell, *et al.,* 2011; Coles*, et al.,* 2006).

Although the identification of anthelmintic resistance in *N. battus* first emerged over 12 years ago, there are very few publications regarding its progression, particularly across Ireland. Therefore, it can be concluded from the limited data obtained, *N. battus* is a genus in which anthelmintic resistance is seldomly reported across Ireland. In comparison, the data obtained from the literature for the resistance status of *N. battus* across the UK concluded that the BNZ anthelmintic drug was the least effective. According to Jones, *et al.,* (2012), it can be expected to identify BNZ resistance on almost 100% of lowland farms and 83% of upland farms across the UK.

The distribution of anthelmintic resistance and multiple-drug resistance in *N. battus* populations is becoming more prevalent across the United Kingdom, which poses substantial limitations on the viability of small-ruminant production systems. However, the failure to detect anthelmintic resistance in *N. battus* across Ireland suggests that there is a lack of reporting across this species (Keane, *et al.,* 2014).

The use of molecular analysis corresponded to being the most sensitive test in the identification of BNZ resistance in *N. battus* within the UK. In particular, the study conducted by Melville, *et al*., (2020), not only confirmed the presence of resistant single nucleotide polymorphism (SNPs) in the DNA sequence of *N. battus* to the BNZ anthelmintic class, but it also aided in identifying the F167Y SNP at a low frequency in four out of five populations – the first mutation to occur in this nematode species.

In comparison, resistance to the ML anthelmintic group has been less evident as it is less understood. Despite this, one study did confirm ML resistance in *N. battus* populations (McMahon, *et al.*, 2017). This study, illustrated how the mechanisms of resistance can vary among the different anthelmintic drugs (McMahon, *et al.*, 2017; Wolstenholme, *et al.*, 2004). In addition, the study also reported the increase in the number of cases of multiple-drug resistance in nematodes. Here, resistance to the BNZ, and the MLs moxidectin and avermectin were present in 36%, 75% and 50% of flocks tested within the UK.

4.1.3 Anthelmintic efficacy across Ireland and the UK using *in vivo* and *in vitro* methods in gastrointestinal nematodes

There is a high variability in the efficacy of the *in vivo* and *in vitro* methods in detecting anthelmintic resistance among the pleathora of parasitic nematodes. There is a need for better, sensitive, diagnostic methods going forward for the detection of anthelmintic resistance. Although the FECRT is considered the practical gold standard for assessing anthelmintic efficacy, there are a number of factors that can infere with the effectiveness of the test, as observed in the study conducted by Ramünke*, et al.,* (2016). It was concluded that the FECRT was limited to only detecting one parasite genus, and its sensitivity was not as effective in detecting BNZ resistance in *Haemonchus, Teladorsagia* and *Trichostrongylus* when compared to the egg hatch assay (EHA) and molecular analysis. The results obtained from the FECRT varies considerably, and are highly dependant on the nematode species under analyses. The use of FECRTs are generally not reliable for the study of acute nematodirosis infections as the worm burdens are typically reduced in the host due to hypersensitivity reactions, thus, making it difficult to differentiate if the reduction in egg counts is due to anthelmintic efficacy or the immune repsonse of the host (McRae, *et al.,* 2015). Similarly, this test is typically only sensitive where approximately 25% of the population of nematodes are resistance to the anthelmintic drug being analysed (Coles, *et al.,* 2006).

From these studies, it can be suggested that the use of the larval development assay (LDA), controlled efficacy test (CET) and the larval migration inhibition assay (LMIA) were efficacious in detecting multiple-drug resistance. Additionally, the use of these tests contributed to the first confirmed case of ivermectin resistance in GIN in sheep in Ireland (Keegan, *et al.,* 2015).

Despite the FECRT being commonly used, the use of molecular analysis has become commonplace. The data obtained from the literature based on the use of molecular analysis provided more efficacious results for establishing anthelmintic resistance. In particular, the most recent study conducted by Melville, *et al.,* (2020), identifed resistant SNPs associated with BNZ resistance in *N. battus* as well as establishing the first case of early emergence of the mutation F167Y in this GIN. However, it has been suggested that the reciprocity between the efficacy of anthelmintics and multiple SNPs may vary, thus, giving rise to inconsistent phenotypic effects (Kotze, *et al.,* 2020). However, the use of these tests provide rapid and sensitive results, yet, they are typically only used as research tools.

4.2 Survey of worming practice and perception of anthelmintic resistance

The survey yielded results from various counties across Ireland and the UK in lowland, upland, and hill areas. It is clear that poor management and nematode control practices have been identified, with many farms not adopting the SCOPS guidelines, which are aimed to slow the development of anthelmintic resistance. Based on these results, there are a number of cofounding factors which may be contributing and influencing the rate of anthelmintic resistance on farms across Ireland and the UK, including: incorrection calculation of dose, treatment frequency, sporadic anthelmintic drug rotation and a lack of calibrating dosing equipment. It is critical that there is more emphasis on the need for improving worming strategies on sheep farms.

5. Conclusions

In conclusion, this study suggests that the intensive use of anthelmintics has led to widespread resistance to a number of broad-spectrum anthelmintics. With few new anthelmintics on the market, and the epidemiology of parasites evolving, the fight against GINs continues. The results from the survey provide mounting evidence that poor nematode control strategies are still being implemented on farms across Ireland and the UK. In order to combat this challenge, it is critically important to understand the management practices associated with anthelmintic resistance. These factors must be used in conjunction with effective monitoring strategies, yet the *in vivo* methods that are readily applied to establish anthelmintic resistance are unsatisfactory due to their lack of sensitivity and reliability. Without effective worm control plans and the use of effective *in vivo* and *in vitro* monitoring tests, animal health and anthelmintic efficacy will continue to deteriorate.

6. Future Work

Based on the findings in this study, it is clear that continuous and comprehensive surveillance of resistance should be carried out. It can be acknowledged that there is a substantial gap in the literature on the prevalence and epidemiology of GIN, namely *N. battus*. In particular, there is a lag between published data across the North and South of Ireland. Hence, by providing clearer evidence on the prevalence and epidemiology of GIN and the changes in hatching stimuli, these factors could aid in lessening the pressure imposed by nematode adaptation. Secondly, more research is required on the comparison of the *in vivo* and *in vitro* methods that are used to establish anthelmintic efficacy. Without the use of effective assays, the fight against anthelmintic resistance is impeded. Finally, more emphasis is needed on accentuating more education and awareness to flock owners on anthelmintic drug choices, administration, and the importance of vigilance in monitoring strategies for GIN. If such measures are implemented, anthelmintic resistance should not progress in *N. battus* to the same extent as other GIN.

7. Acknowledgements

I would like to thank my academic supervisor, Dr. Lisa Geraghty at the Technological University of the Shannon, whose expertise has been extremely valuable throughout the duration of this project. In particular, I want to thank you for your ongoing input and guidance throughout. Secondly, I would like to thank the Technological University of the Shannon for the use of the facilities during my time spent here.

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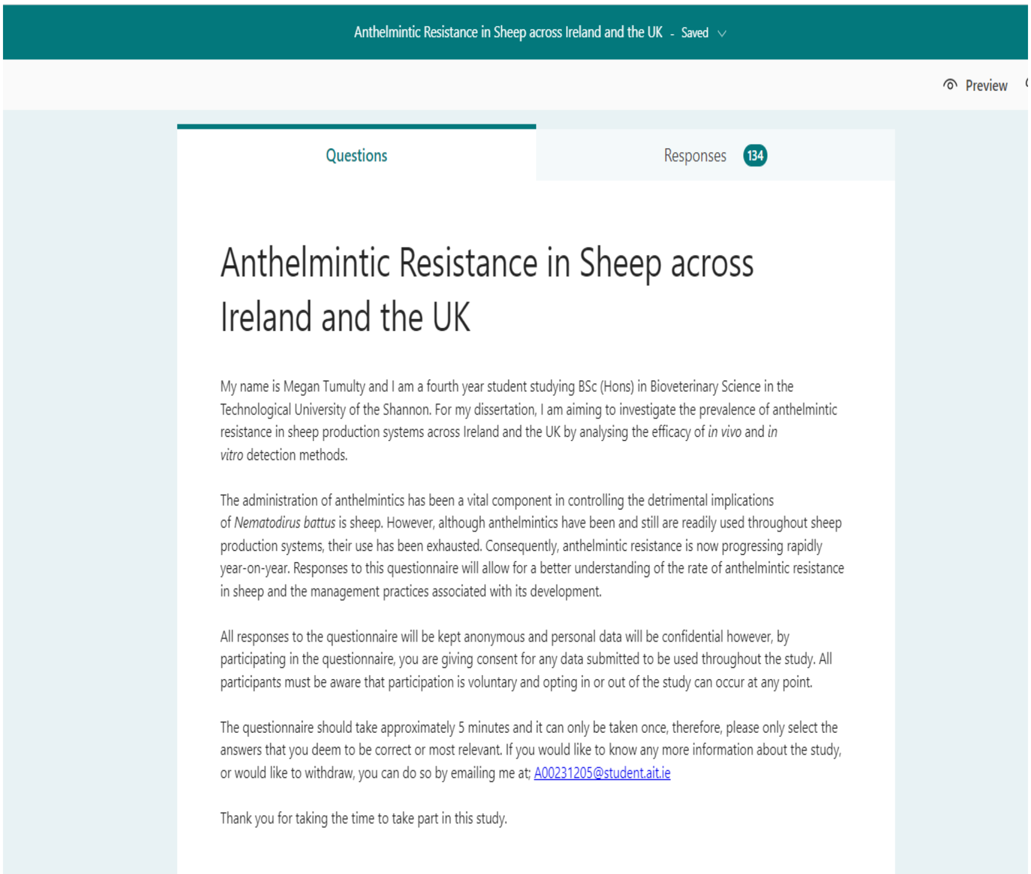
9. Appendix

Appendix 1: Survey distributed to farmers across Ireland and the UK



Anthelmintic Resistance in Sheep across Ireland and the UK - Saved

Preview

Questions

Responses 134

Anthelmintic Resistance in Sheep across Ireland and the UK

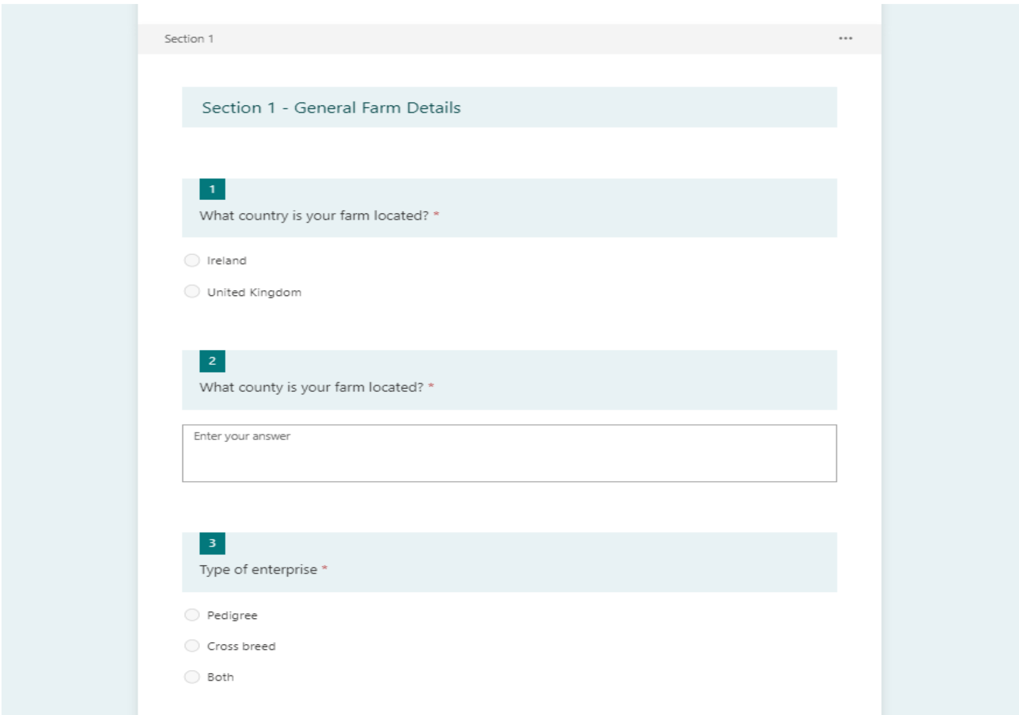
My name is Megan Tumulty and I am a fourth year student studying BSc (Hons) in Bioveterinary Science in the Technological University of the Shannon. For my dissertation, I am aiming to investigate the prevalence of anthelmintic resistance in sheep production systems across Ireland and the UK by analysing the efficacy of *in vivo* and *in vitro* detection methods.

The administration of anthelmintics has been a vital component in controlling the detrimental implications of *Nematodirus battus* is sheep. However, although anthelmintics have been and still are readily used throughout sheep production systems, their use has been exhausted. Consequently, anthelmintic resistance is now progressing rapidly year-on-year. Responses to this questionnaire will allow for a better understanding of the rate of anthelmintic resistance in sheep and the management practices associated with its development.

All responses to the questionnaire will be kept anonymous and personal data will be confidential however, by participating in the questionnaire, you are giving consent for any data submitted to be used throughout the study. All participants must be aware that participation is voluntary and opting in or out of the study can occur at any point.

The questionnaire should take approximately 5 minutes and it can only be taken once, therefore, please only select the answers that you deem to be correct or most relevant. If you would like to know any more information about the study, or would like to withdraw, you can do so by emailing me at; A00231205@student.ait.ie

Thank you for taking the time to take part in this study.



Section 1 ...

Section 1 - General Farm Details

1
What country is your farm located? *

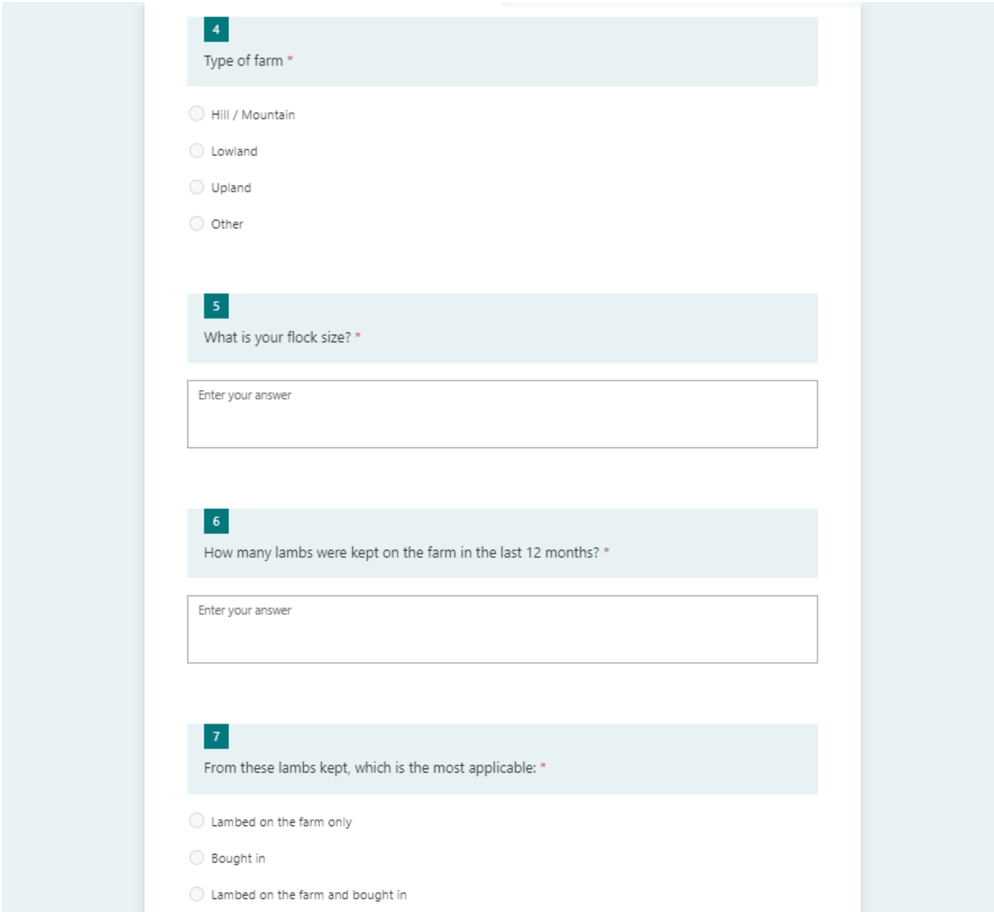
- Ireland
- United Kingdom

2
What county is your farm located? *

Enter your answer

3
Type of enterprise *

- Pedigree
- Cross breed
- Both


4
Type of farm *

- Hill / Mountain
- Lowland
- Upland
- Other

5
What is your flock size? *

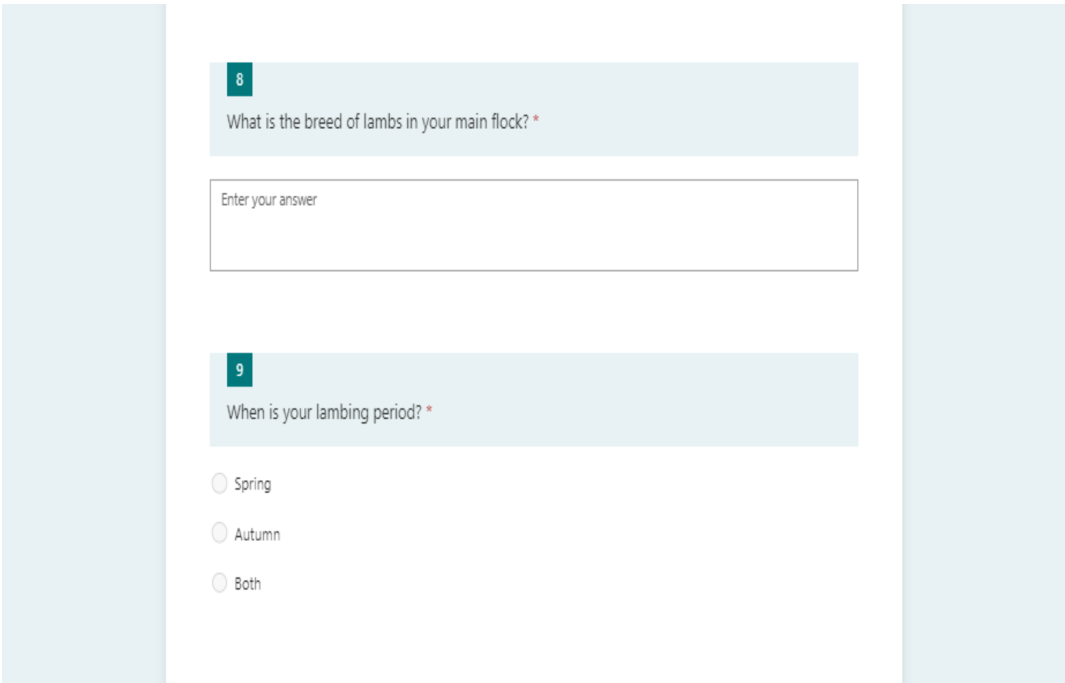
Enter your answer

6
How many lambs were kept on the farm in the last 12 months? *

Enter your answer

7
From these lambs kept, which is the most applicable: *

- Lambed on the farm only
- Bought in
- Lambed on the farm and bought in


8
What is the breed of lambs in your main flock? *

Enter your answer

9
When is your lambing period? *

- Spring
- Autumn
- Both

Section 2 - Worming Regime and Grazing Management

10

Are roundworms a problem for lambs on your farm? *

- Yes
- No
- I don't know

11

Do you administer wormers to lambs to control roundworms? *

- Yes
- No

12

How is the decision made to treat lambs for worms? *

- Weather forecast
- Faecal egg count
- Same time each year
- Presence of ill-thrift (e.g. scouring)
- Advice from veterinarian
- Advice from other farmers
- Pre/post lambing
- Other

13

Are all lambs treated, or is only a percentage treated? *

- 100% of lambs are treated
- Less than 100% of lambs are treated

14

How is the volume of drench determined? *

- A rough estimate
- Group average
- Heaviest in the group
- Weigh individual

15
Is the dosing equipment checked before use? *

- Always
- Sometimes
- Never

16
Is the "best before" date checked before use? *

- Always
- Sometimes
- Never

17
How is treatment failure followed up? *

- Faecal egg count reduction test
- Veterinary advice
- Not followed up
- Other

18
In your opinion, have you experienced any anthelmintic resistance in your flock within the **last 5 years**? *

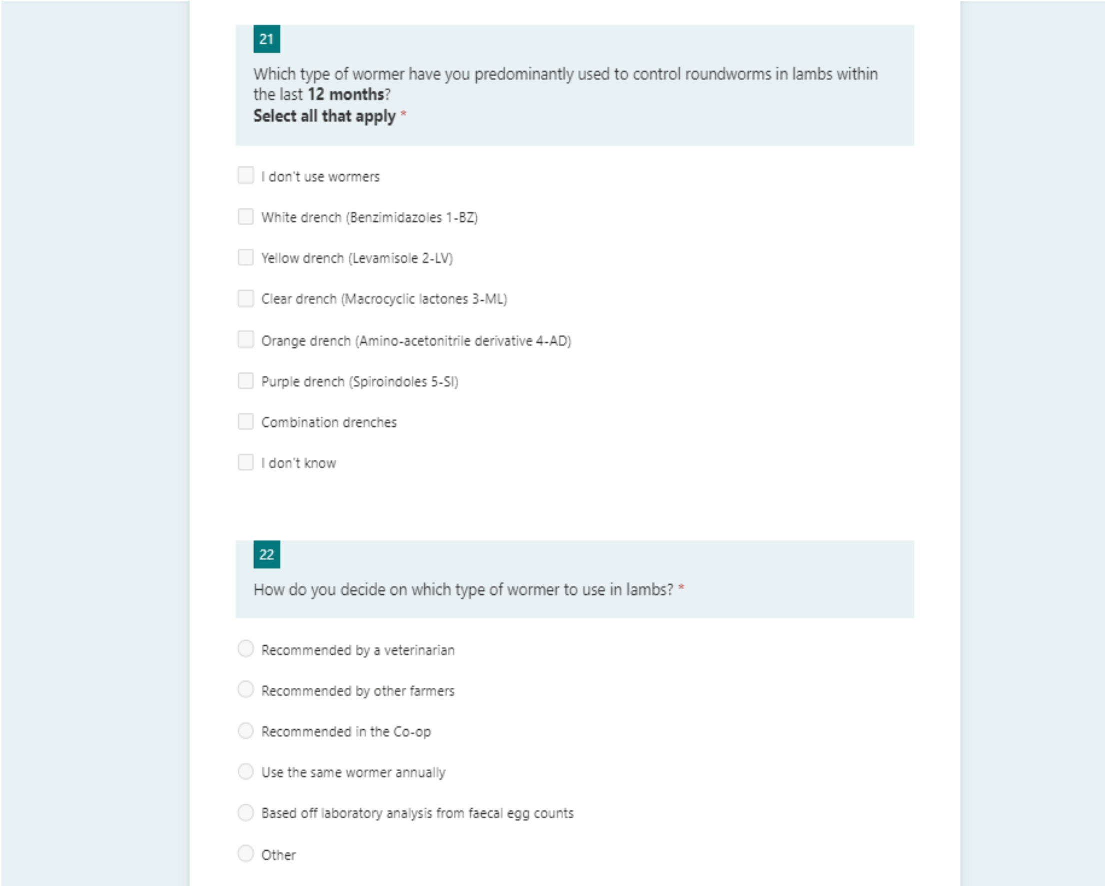
- Yes
- No
- I don't know

19
Are lambs moved to a clean pasture for a few days **before** dosing with a wormer? *

- Yes
- No
- Sometimes

20
Are lambs moved to a clean pasture directly **after** dosing with a wormer? *

- Yes
- No
- Sometimes



21

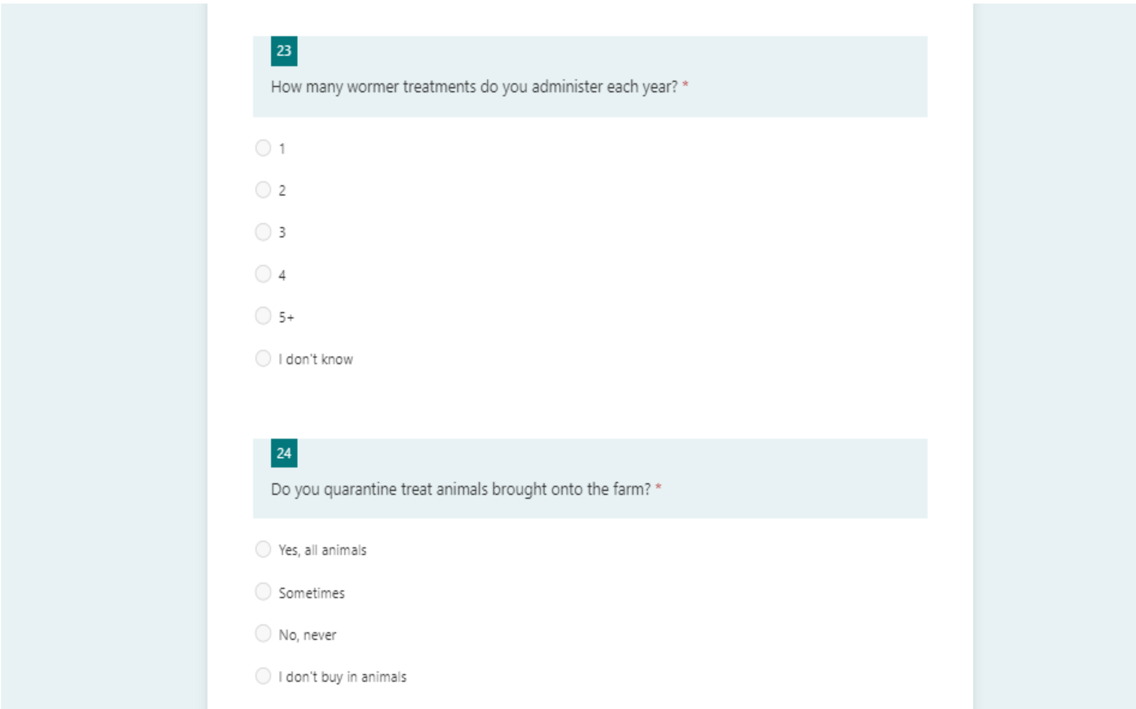
Which type of wormer have you predominantly used to control roundworms in lambs within the last **12 months**?
Select all that apply *

- I don't use wormers
- White drench (Benzimidazoles 1-BZ)
- Yellow drench (Levamisole 2-LV)
- Clear drench (Macrocyclic lactones 3-ML)
- Orange drench (Amino-acetonitrile derivative 4-AD)
- Purple drench (Spiroindoles 5-SI)
- Combination drenches
- I don't know

22

How do you decide on which type of wormer to use in lambs? *

- Recommended by a veterinarian
- Recommended by other farmers
- Recommended in the Co-op
- Use the same wormer annually
- Based off laboratory analysis from faecal egg counts
- Other



23

How many wormer treatments do you administer each year? *

- 1
- 2
- 3
- 4
- 5+
- I don't know

24

Do you quarantine treat animals brought onto the farm? *

- Yes, all animals
- Sometimes
- No, never
- I don't buy in animals

25

Which type of wormer do you use during quarantine?
Select all that apply *

- I don't use wormers
- White drench (Benzimidazoles 1-BZ)
- Yellow drench (Levamisole 2-LV)
- Clear drench (Macrocyclic lactones 3-ML)
- Orange drench (Amino-acetonitrile derivative 4-AD)
- Purple drench (Spiroindoles 5-SI)
- Combination drenches
- I don't know

26

Do you treat lambs for *Nematodirus* (gastrointestinal worm) infection? *

- Yes
- No
- I don't know

27

Have you had a *Nematodirus* infection on your farm within the last **5 years**? *

- Yes, high level infection (scouring and death)
- Yes, low level infection (scouring, ill thrift but no death)
- Yes, but no signs of infection
- No, no signs of infection
- I don't know

28

If so, when do lambs generally show clinical signs of *Nematodirus* infection?

- Spring (March-May)
- Summer (June-August)
- Autumn (September-November)
- Winter (December-February)

29

Do you supplement lambs with feed? *

- Creep feed pre-weaning
- Ad lib post weaning
- No supplementary feeding
- Other